



## SOME BACTERIOLOGICAL STUDIES OF CLAW AFFECTIONS IN CATTLE IN ASSIUT GOVERNORATE

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### ABSTRACT :

A number of 400 Friesian cows were inspected clinically for claw affections. The incidence of affections was 13.75%, hind claw affection (38) were more frequent than those of fore claw (17). Bacteriological examination of aseptically collected tissue samples from affected claws revealed several aerobic and anaerobic organisms in a descending manner either in pure or mixed culture. *Fusobacterium necrophorum* (*F.necrophorum*) was the most commonly isolated anaerobic bacteria and *Corynebacterium pyogenes* (*C.pyogenes*) was the most commonly isolated aerobic species with isolation rate of 67.27% and 52-73% respectively. The virulence of *F.necrophorum* in mice was related to the route of infection and strain biotype. The *in vitro* sensitivity of the main recovered pathogenic isolates to 10 antimicrobial agents was described in details.

### INTRODUCTION :

Skin lesions of cows' feet have been reported as early as the beginning of the 19<sup>th</sup> century. There are five skin lesions of bovine claw recognised today which are associated with infectious agents, namely digital dermatitis, interdigital dermatitis, interdigital necrobacillosis, peracute foul and dermatitis verrucosa (Demirkan *et al.*, 2000). In this context the natural environment of the bovine foot may play a major role in these skin infections (Moustafa, *et al.*, 1994 b).

There is some heresay evidence to suggest that claw trimmers using dirty hoof knives can transfer microorganisms associated with infectious causes of specific skin diseases of bovine claw from farm to farm or from infected

to healthy susceptible cows (Demirkan, *et al.*, 2000).

Severe complications may occur in untreated cases resulting in malformation of the claws and permanent lameness (Egerton, 1979).

Treatment of lesions are an expensive and laborious task. In order to reduce the losses to the cattle industry, it is necessary to ascertain the exact cause of skin diseases of bovine claw and to develop a satisfactory means of prevention and treatment (Gupta, *et al.*, 1964).

The present work investigated the bacterial strains isolated from lesions of bovine claw, biotyping of *F.necrophorum* isolates, and application of experimental infection of *F.necrophorum* in mice. In addition it aimed to study the antibiograms of the most commonly isolated organisms.

## **MATERIAL AND METHODS :**

### **I-Collection of Specimens:**

In the present study 400 Friesian cows, from different localities in Assiut Governorate namely governmental and private sector farms, were inspected. The age of the examined animals ranged from 2-8 years. Out of the examined animals 55 had signs of claw affections (foot rot lesions at various stages of development). The lesion was accurately diagnosed by location, fetid odor and a characteristic swelling of the interdigital area and the bulbs of the heels. Prior to sample collection from the hooves of clinically affected animals, the feet were washed to remove soil and faecal matters. Tissue samples were taken from the affected areas by using sterile scalpel and forceps. Collected tissue samples were rapidly transported to the laboratory without undue delay where the samples were bacteriologically examined.

### **II- Experimental Techniques:**

#### **1-Isolation and identification of aerobic and anaerobic organisms :**

Each tissue sample was divided into two portions. The first portion was examined for aerobic organisms on the basis of Koneman *et al.*, (1992) and Quinn *et al.*, (1994) using the following media: nutrient agar, sheep blood agar, MacConkey's agar and mannitol salt agar. Identification of the different isolates was achieved mainly on the basis of their morphology, culture characteristics and biochemical reactions. While the second portion was used for detection of anaerobic organisms, where it was inserted in a tube of thioglycolate medium. The inoculated tubes were incubated at 37°C for 24 hours in Gas pak jar using Gas-pak envelop (BBL, Becton Dickinson

Microbiology System. Cockey Sville, MD 21030 U. S. A.). Then, subcultures were streaked onto the surface of blood agar medium (Koneman *et al.*, 1992). The inoculated plates were incubated under condition mentioned above, for 3 days. Suspected colonies were identified according to Smith and Holdeman (1968). Isolates that produced biochemical reactions simulating *F.necrophorum* were subjected to further identification according to the characteristics detailed by Fievez (1963) and Moore and Holdeman (1974). *F.necrophorum* was differentiated from other Fusobacteria by its production of lipase when tested on egg-yolk agar (Koneman *et al.*, 1992).

#### **2-Pathogenicity of *F.necrophorum* to mice :**

##### **a-Strains for investigation :**

Six isolates of biochemically identified *F. necrophorum* isolated in this study were selected for pathogenicity studies; three of biotype (A) and the other three of biotype (AB).

##### **b-Laboratory animals.**

Swiss male mice, each of approximately 20 gm weight were used. The mice were obtained from the Animal-house, Assiut University. Three mice were used for each strain (two inoculated with the organism and one with sterile broth and left as a control). Mice used for experimental infection were divided into 2 groups and a third group was kept as a control group. The first group of mice was injected intraperitoneally (I/P) and the second group was injected subcutaneously (S/C). The inocula for *in vivo* studies and the animal inoculation technique were prepared as described by Maestrone *et al.*, (1975). All the inoculated and control mice were kept under observation. The number of dead mice were recorded.



### 3-Antimicrobial susceptibility testing:

The predominant aerobic and anaerobic isolates obtained in this study were tested for antimicrobial susceptibility by disc diffusion method as described by Finegold and Martin (1982) for aerobic bacteria and as under strict anaerobic conditions described by Maestrone *et*

*al.*, (1975) and Collee *et al.*, (1989) for anaerobic isolates using ten antimicrobial agents.

### RESULTS :

The results are tabulated in Tables 1,2,3,4,5 and 6.

Table (1): Incidence of claw affections in Friesian cows.

| Species       | No. of inspected animals | No. of affected cases  |                        |       | Incidence percentage |
|---------------|--------------------------|------------------------|------------------------|-------|----------------------|
|               |                          | Claws of the hind feet | Claws of the fore feet | Total |                      |
| Friesian cows | 400                      | 38                     | 17                     | 55    | 13.75                |

Table (2): Prevalence rate of aerobic bacteria recovered from bovine claw lesions.

| Aerobic isolates                | No. of isolates | Percentage* |
|---------------------------------|-----------------|-------------|
| <i>Corynebacterium pyogenes</i> | 29              | 52.73       |
| <i>Staphylococcus aureus</i>    | 24              | 43.64       |
| <i>Escherichia coli</i>         | 21              | 38.18       |
| <i>Proteus vulgaris</i>         | 13              | 23.64       |
| <i>Citrobacter diversus</i>     | 11              | 20          |
| <i>Pseudomonas aeruginosa</i>   | 9               | 16.36       |
| <i>Salmonella spp.</i>          | 6               | 10.91       |
| <i>Nocardia spp.</i>            | 4               | 7.27        |

\*The percentage was calculated according to the total number of examined samples (55 specimens).

Table (3): Prevalence rate of strict anaerobic bacteria recovered from bovine claw lesions.

| Anaerobic isolates                         | No. of isolates | Percentage* |
|--|-----------------|-------------|
| <i>Fusobacterium necrophorum</i>           | 37              | 67.27       |
| <i>Dichelobacter (Bacteriodes) nodosus</i> | 14              | 25.45       |
| <i>Peptostreptococcus anaerobes</i>        | 10              | 18.18       |
| Unidentified <i>Clostridium spp.</i>       | 3               | 5.45        |

\*The percentage was calculated according to the total number of examined samples (55 specimens).

**Table (4): Differentiation of *F.necrophorum* biotypes isolated from bovine claw affections.**

| Reactions                     | Type A (28) | Type AB (9) |
|-------------------------------|-------------|-------------|
| Haemagglutination             | +           | ±           |
| Haemolysis                    | +           | +           |
| Pathogenicity to mice         | +++         | ++          |
| Sedimentation in liquid broth | -           | ±           |
| Production of lipase          | +           | +           |

+++ = Highly pathogenic for mice.

++ = Moderately pathogenic for mice.

+ = More than 84% of strains were positive.

± = Between 50-84% of strains were positive.

- = Less than 10% of strains were positive.

**Table (5): pathogenicity of *F.necrophorum* strains for mice by subcutaneously (S/C) and intraperitoneal (I/P) routes of inoculation**

| <i>F.necrophorum</i> strains | No. of strains examined | No. of mice injected | No. of control mice | Routes of injection                     |                |   |                |
|------------------------------|-------------------------|----------------------|---------------------|---|----------------|---|----------------|
|                              |                         |                      |                     | S/C                                     |                | I/P                                     |                |
|                              |                         |                      |                     | No. of mice died/No. of mice inoculated | Mortality rate | No. of mice died/No. of mice inoculated | Mortality rate |
| Biotype (A)                  | 3                       | 6                    | 3                   | *5/6**                                  | 83.33%         | *6/6**                                  | 100%           |
| Biotype (AB)                 | 3                       | 6                    | 3                   | *1/6**                                  | 16.67%         | *4/6**                                  | 66.67%         |

\* No. of mice died.

\*\* No. of mice inoculated.

## DISCUSSION :

For foot rot to occur in cattle, an interaction seems to be essential between etiological agent (s), the host resistance, the type of management practice and other environmental factors.

Incidence percentage of claw affections (Foot rot lesions at various stages of development) in the present study was 13.75% in Friesian cows (Table 1). A higher incidence (25.71%) was reported by El-Sayed Enany *et al.*, (1994). Marshy, filthy and wet surroundings as well as concrete flooring and coarse sand predispose the animal to the disease (Gupta *et al.*, 1964). It was noted that the claws of the hind feet had more lesions than the claws of the fore ones in Friesian cows (Table 1). This conclusion substantiates what have been reported by Cygan *et al.*, (1977) and Nuttler and Moffitt (1990). It appears that such phenomenon might be the result of the subjection of the hind feet to

moisture due to the presence of urine and faeces especially in illdrained stalls. Wet mud and faeces often soften and macerate the interdigital skin, facilitating entry of the pathogens which are in abundance in such materials. (Gilder, 1960).

The present work was planned to throw some light on the possible bacterial agents which may be incriminated in bovine claw affections. Concerning aerobic microorganisms, the following species were isolated in a descending manner: *Corynebacterium pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Proteus vulgaris*, *Citrobacter diversus* *Pseudomonas. aeruginosa*, *Salmonella spp.*, and *Nocardia spp.* (Table 2). Several authors in Egypt and allover the world reported the isolation of aerobic bacteria from bovine claw affections (Gupta *et al.*, 1964; Cygan *et al.*, 1977; Samy *et al.*, 1984; Mohamed, 1988 and El-Sayed Enany *et al.*, 1994).

**Table (6): In vitro antimicrobial drug sensitivity of the predominant anaerobic and aerobic bacteria isolated from bovine claw affections.**

| Anti bacterial agent<br>Content/disc                     | Anaerobic bacteria           |      |    |      |                          |      |    |      | Aerobic bacteria           |      |    |      |                          |      |    |      |                        |      |    |      |
|--|------------------------------|------|----|------|--------------------------|------|----|------|----------------------------|------|----|------|--------------------------|------|----|------|------------------------|------|----|------|
|  | <i>F.necrophorum</i><br>(37) |      |    |      | <i>D.nodosus</i><br>(14) |      |    |      | <i>C. pyogenes</i><br>(29) |      |    |      | <i>S. aureus</i><br>(24) |      |    |      | <i>E. coli</i><br>(21) |      |    |      |
|  | S                            |      | R  |      | S                        |      | R  |      | S                          |      | R  |      | S                        |      | R  |      | S                      |      | R  |      |
|  | No                           | %    | No | %    | No                       | %    | No | %    | No                         | %    | No | %    | No                       | %    | No | %    | No                     | %    | No | %    |
| Penicillin G (10 IU)                                     | 37                           | 100  | 0  | 0.00 | 14                       | 100  | 0  | 0.00 | 29                         | 100  | 0  | 0.00 | 11                       | 45.8 | 13 | 54.2 | 0                      | 0.00 | 21 | 100  |
| Danofloxacin (5µg)                                       | 31                           | 83.8 | 6  | 16.2 | 13                       | 92.9 | 1  | 7.14 | 11                         | 37.9 | 18 | 62.1 | 6                        | 25   | 18 | 75.0 | 20                     | 95.2 | 1  | 4.76 |
| Enrofloxacin (10µg)                                      | 23                           | 62.2 | 14 | 37.8 | 11                       | 78.6 | 3  | 21.4 | 8                          | 27.6 | 21 | 72.4 | 5                        | 20.8 | 19 | 79.2 | 6                      | 28.6 | 15 | 71.4 |
| Trimethoprim “ Sulphame-<br>thoxazol (1.25 µg + 23.75µg) | 21                           | 56.8 | 16 | 43.2 | 10                       | 71.4 | 4  | 28.6 | 16                         | 55.2 | 13 | 44.8 | 20                       | 83.3 | 4  | 16.7 | 11                     | 52.4 | 10 | 47.7 |
| Rifampicin (30 µg)                                       | 20                           | 54.1 | 17 | 45.9 | 9                        | 64.3 | 5  | 35.7 | 29                         | 100  | 0  | 0.00 | 24                       | 100  | 0  | 0.00 | 17                     | 80.9 | 4  | 19.1 |
| Chloramphenicol (30µg)                                   | 34                           | 91.9 | 3  | 8.11 | 13                       | 92.9 | 1  | 7.14 | 29                         | 100  | 0  | 0.00 | 24                       | 100  | 0  | 0.00 | 18                     | 85.7 | 3  | 14.3 |
| Erythromycin (15 µg)                                     | 29                           | 78.4 | 8  | 21.7 | 7                        | 50   | 7  | 50.0 | 21                         | 72.4 | 8  | 27.6 | 22                       | 91.7 | 2  | 8.33 | 9                      | 42.9 | 12 | 57.2 |
| Streptomycin (10 µg)                                     | 3                            | 8.11 | 34 | 91.9 | 0                        | 0.00 | 14 | 100  | 0                          | 0.00 | 29 | 100  | 0                        | 0.00 | 24 | 100  | 0                      | 0.00 | 21 | 100  |
| Colistin-sulphate (10µg)                                 | 0                            | 0.00 | 37 | 100  | 0                        | 0.00 | 14 | 100  | 12                         | 41.4 | 17 | 58.6 | 9                        | 37.5 | 15 | 62.5 | 0                      | 0.00 | 21 | 100  |
| Kanamycin (30 µg)  | 36                           | 97.3 | 1  | 2.70 | 13                       | 92.9 | 1  | 7.14 | 0                          | 0.00 | 29 | 100  | 14                       | 58.3 | 10 | 41.7 | 11                     | 52.4 | 10 | 47.7 |

S = Sensitive R = Resistant

As regards the anaerobic bacteria, the results given in Table (3) revealed that *F.necrophorum* was the most prevalent species, with an isolation rate of 67.27%. This result is somewhat similar to those reported by Gupta *et al.*, (1964) and Radwan (1999) who isolated *F.necrophorum* from 25 (62.5%) and 62 (62%) out of 40 and 100 samples from cows suffering from interdigital dermatitis. On the other hand, El-Sayed Enany *et al.*, (1994) and Togoe *et al.*, (1995) reported higher incidences of 76.67% and 82.5% respectively. On the other hand, Moustafa *et al.*, (1994 a) reported a lower incidence of 44.44%. It had been argued that trauma or devitalisation of tissue such as that caused by rumenal acidosis or penetrating lesions of the foot is a prerequisite for colonisation by *F.necrophorum* (Scanlan and Hathcock, 1983). Furthermore, *F.necrophorum* synthesizes a protein, Leukocidin, which would destroy cell membranes and increase the inflammatory response (Kanoë *et al.*, 1986).

37 strains of *F.necrophorum* were isolated from bovine claws suffering from foot rot lesions at various stages of development. 28 out of these isolates were identified as being biotype (A) and the remaining nine were belonged to biotype (AB) (Table 4). The present classification was based on their biochemical characteristics. The biotypes of *F.necrophorum* have differences in cellular and colony morphology and are identified by their biological characteristics. The present results are somewhat similar to those reported by Kanoë *et al.*, (1978) and Scanlan and Hathcock (1983) who found that *F.necrophorum* strains recovered from claw lesions of bovine feet were generally of biotype A and AB.

Information derived from Tables (2& 3) declares that *F.necrophorum* and *C.pyogenes* were the most commonly isolated aerobic and

anaerobic species from claw lesions. *C. pyogenes* produces a diffusible factor that stimulates the growth of *F.necrophorum* and also it lowers the partial pressure of oxygen and oxidation-reduction potential in the infected tissues (Roberts, 1967 a). On the other hand, *F.necrophorum* produces a leukotoxin that protects both itself and *C.pyogenes* from phagocytosis (Roberts, 1967 b).

It is evidence from Table (3), that the recovery rate of *Dichelobacter (Bacteriodes) nodosus (D.nodosus)* was 25.45%. *D.nodosus* was isolated by Bolte *et al.*, (1990) from 72% of cases involving acute dermatitis in the interdigital space of cattle. However, El-Sayed Enany *et al.*, (1994) noticed that 37.78% of foot rot specimens taken from cattle harboured *D.nodosus*. Moreover, Radwan (1999) recovered 3 species of genus *Bacteriodes* and found that only 5 foot rot specimens taken from cows contained *D.nodosus*. On the other hand, the results of the present study were disagreed with that reported by Gupta *et al.*, (1964) and Mohamed (1988) who did not find *D.nodosus* in foot-rot samples taken from cattle.

All *D.nodosus* strains recovered in this study were in combination either with biotype A or AB of *F.necrophorum*. These findings substantiate those reported by Scanlan (1990) who reported that *D.nodosus* had been isolated in cases of foul-in-foot in association with *F.necrophorum*.

Information derived from Table (3) revealed that a Gram negative anaerobes (*F.necrophorum* and *D.nodosus*) were the most prevalent anaerobic bacteria such phenomenon might be due to the bovine foot has anatomic features that may contribute to its susceptibility to infection by a Gram negative anaerobes (Moustafa *et al.*, 1994 a).

Other anaerobic bacteria were isolated and they included *Peptostreptococcus anaerobes* (*P.anaerobes*) and unidentified clostridium species with an incidence of 18.18% and 5.45% respectively. Some authors like El-Sayed Enany *et al.*, (1994) and Radwan (1999) recorded very high incidences of the same organisms as compared with those of the present results.

Pathogenicity of *F.necrophorum* for the white mouse:

The data obtained using I/P and S/C routes of inoculation as shown in Table (5), indicated that the pathogenicity was related to strain biotypes, since biotype A strains were more virulent than biotype AB strains. On the other hand, the pathogenicity of *F.necrophorum* in mice was related to the route of inoculation. *F. necrophorum* biotype A injected I/P and S/C caused mortality rates of 100% and 83.33% respectively while those of biotype AB were 66.67% and 16.67% respectively. This result goes hand in hand with those reported by Fievez, (1963); Maestrone *et al.*,(1975) and Radwan (1999). A contradictory finding was given by El-Sayed Enany *et al.*, (1994) who reported the death of all injected swiss-mice through either routes.

The early deaths were observed 4 days after I/P inoculation and became more prominent after 7 days, while the death following the S/C route was within 11-15 days post inoculation. The gross post mortem picture similar to that previously recorded in *F.necrophorum* infection in mice by Maestrone *et al.* (1975) and Radwan (1999). little is known about the pathogenesis of *F.necrophorum* in adult mice but pathological changes and deaths among mice are probably attributed to Fusobacterium lipopolysaccharide which may play an important role in initiating pathological changes associated with infectious dermatitis (Kanoë *et al.*, 1995).

From the above findings it is concluded that biotype A strains of *F.necrophorum* was more pathogenic than AB ones and this may be attributed to the luxuriant production of leukotoxin by biotype A while the biotype AB produced slightly less leukotoxin. This conclusion substantiates what had been reported by Coyle-Dennis and Lauerman (1979) as they demonstrated that a leukotoxin producing strain was more pathogenic to mice than was a non-leukotoxin producing strain.

As regards the *in vitro* sensitivity of the predominant aerobic bacteria isolated from claw affections (Table 6), it is evident that Penicillin-G, Rifampicin and Chloramphenicol were the most effective antibiotics against *C.pyogenes* and *S.aureus* at a rate of 100%. The majority of *E. coli* strains, were sensitive to Rifampicin (80.95%), Chloramphenicol (85.71%) and Danofloxacin (95.24%). These findings agree to a certain extent with those reported by Mohamed (1988) and El-Sayed Enany *et al.*, (1994).

The antibiogram of the predominant isolates of anaerobic bacteria showed that Penicillin-G was the most effective antibiotic against *F.necrophorum* and *D.nodosus* at a rate of 100%, and they also showed a significantly high degree of sensitivity to Kanamycin and Danofloxacin (Table 6).

From above results it is revealed that anaerobic and most aerobic bacteria associated with claw affections were 100% sensitive to Penicillin-G. This conclusion substantiates what had been reported by Annaheim (1964) who found that parenteral treatment with Penicillin-G alone was effective only in the early phase of the disease.

Finally, it is concluded that the *in vitro* sensitivity of the isolated strains is of great importance to choose the most effective drug for



treating and controlling such economic problem.

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## بعض الدراسات البكتريولوجية عن إصابة الأظلاف فى الأبقار فى محافظة أسيوط

صديق رشوان صديق ، الفونس فخرى بسطاوروس ، نبيل حبيب مقار

تم فحص عدد ٤٠٠ بقرة اكلينيكيا لاستبيان وجود إصابات فى الأظلاف واتضح أن نسبة الإصابة ١٣,٧٥% وكانت إصابات الأظلاف أكثر حدوثا فى الأظلاف الخلفية (٣٨ ظلف مصاب) عنها فى الأظلاف الأمامية (١٧ إصابة) فى الأبقار الفريزيان .

وأظهر الفحص البكتريولوجى للعينات المأخوذة من الأظلاف المصابة العديد من الميكروبات الهوائية واللاهوائية فى تسلسل تنازلى بصورة نقية أو مختلطة، ولقد وجد أن بكتريا الفيوزوباكتريم نيكروفوريم (٦٧,٢٧%) ، والكورينى باكتريم بيوجينيس بنسبة (٥٢,٧٣%) هى أكثر الميكروبات اللاهوائية والهوائية التى تم عزلها على الترتيب ، وتمت دراسة مدى ضراوة ميكروبات الفيوزوباكتريم نيكروفوريم المعزولة فى هذه الدراسة على الفرن السويسرية البيضاء ، وأثبت البحث أن ضراوة الميكروب له علاقة بطريقة الحقن ونوع العترة المصنفة بيوكيميائياً ، كما تم إجراء اختبار الحساسية للمعزولات الأكثر عزلاً فى هذه الدراسة ضد ١٠ أنواع من المضادات الحيوية.